AMENDMENTS TO THE SPECIFICATION

Please replace the second paragraph at page 1, lines 17 to 32, with the following amended paragraph:

Molecules related to protein Z, derived from domain B of staphylococcal protein A (SPA) (Nilsson B et al (1987) Protein Engineering 1, 107-133), have been selected from a library of randomized such molecules using different interaction targets (see e g WO95/19374; Wo00/63243; Nord K et al (1995) Prot Eng 8:601-608; Nord K et al (1997) Nature Biotechnology 15, 772-777). Different target molecules have been used to select such protein Z derivatives, ~~e g~~ e.g., as described in Nord K et al (1997, supra). The experiments described in this reference outline principles of the general technology of selecting protein Z derivatives against given targets, rather than being a study directed towards the express objective of obtaining a molecule with high enough affinity for use in a specific therapeutic or biotechnological application.

Please replace the paragraph bridging page 7, line 32 through page 8, line 12, with the following amended paragraph:

"Binding affinity for HER2" refers to a property of a polypeptide which may be tested ~~e.g.~~ e.g., by the use of surface Plasmon resonance technology, such as in Biacore® instrument. HER2 binding affinity may be tested in an experiment wherein HER2 is immobilized on a sensor chip of the instrument, and a sample containing the polypeptide to be tested is passed over the chip. Alternatively, the polypeptide to be tested is immobilized on a sensor chip of the instrument, and a sample containing HER2 is passed over the chip. The skilled person may then interpret the sensorgrams obtained to establish at least a qualitative measure of the polypeptide's binding affinity for HER2. If a quantitative measure is sought, ~~e.g.~~ e.g., with the purpose to establish a certain K_D value for the interaction, it is again possible to use surface plasmon resonance methods. Binding values may ~~e.g.~~ e.g., be defined in a Biacore® 2000 instrument (Biacore AB). HER2 is immobilized on a sensor chip of the instrument, and samples of the polypeptide whose affinity is to be determined are prepared by serial dilution and injected in random order. K_D values may then be calculated from the results, using ~~e.g.~~ e.g., the 1:1 Langmuir binding model of the

BIAevaluation 3.2 software provided by the instrument manufacturer.

Please replace the paragraph bridging page 8, line 13, to page 9, line 2, with the following amended paragraph:

As stated above, the sequence of the polypeptide according to the present invention is related to the SPA domain sequence in that from 1 to about 20 amino acid residues of said SPA domain have been substituted for other amino acid residues. However, the substitution mutations introduced should not affect the basic structure of the polypeptide. That is, the overall fold of the C_α backbone of the polypeptide of the invention will be essentially the same as that of the SPA domain to which it is related, ~~e g~~ e.g., having the same elements of secondary structure in the same order etc. Thus, polypeptides fall under the definition of having the same fold as the SPA domain if basic structural properties are shared, those properties ~~e g~~ e.g., resulting in similar CD spectra. The skilled person is aware of other parameters that are relevant. This requirement of essentially conserving the basic structure of the SPA domain, upon mutation thereof, places restrictions on what positions of the domain may be subject to substitution. When starting from

the known structure of the Z protein, for example, it is preferred that amino acid residues located on the surface of the Z protein are substituted, whereas amino acid residues buried within the core of the Z protein "three-helix bundle" should be kept constant in order to preserve the structural properties of the molecule. The same reasoning applies to other SPA domains, and fragments thereof.

Please replace the paragraph at page 9, lines 3-21, with the following amended paragraph:

The invention also encompasses polypeptides in which the HER2 binding polypeptide described above is present as an HER2 binding domain, to which additional amino acid residues have been added at either terminal. These additional amino acid residues may play a role in the binding of HER2 by the polypeptide, but may equally well serve other purposes, related for example to one or more of the production, purification, stabilization, coupling or detection of the polypeptide. Such additional amino acid residues may comprise one or more amino acid residues added for purposes of chemical coupling. An example of this is the addition of a cysteine residue at the very first or very last position in the polypeptide chain, ~~i e~~

i.e., at the N or C terminus. Such additional amino acid resides may also comprise a "tag" for purification or detection of the polypeptide, such as a hexahistidyl (His_6) tag, or a "myc" tag or a "flag" tag for interaction with antibodies specific to the tag. The skilled person is aware of other alternatives.

Please replace the paragraph bridging page 10, line 12, to page 11, line 17, with the following amended paragraph:

Additionally, "heterogenic" fusion polypeptides, in which the HER2 binding polypeptide constitutes a first domain, or first moiety, the second and further moieties have other functions than binding HER2, are also contemplated and fall within the ambit of the present invention. The second and further moiety/moieties of the fusion polypeptide may comprise a binding domain with affinity for another target molecule than HER2. Such a binding domain may well also be related to an SPA domain through substitution mutation at from 1 to about 20 positions thereof. The result is then a fusion polypeptide having at least one HER2-binding domain and at least one domain with affinity for said other target molecule, in which both domains are related to an SPA domain. This makes it possible to create multispecific reagents that may be used in several

biotechnological applications, such as used as therapeautic agents or as capture, detection or separation reagents. The preparation of such multispecific multimers of SPA domain related polypeptides, in which at least one polypeptide domain has affinity for HER2, may be effected as described above for the multimer of several HER2 binding "units". In other alternatives, the second or further moiety or moieties may comprise an unrelated, naturally occurring or recombinant, protein (or a fragment thereof retaining the binding capability of the naturally occurring or recombinant protein) having a binding affinity for a target. An example of such a binding protein, which has an affinity for human serum albumin and may be used as fusion partner with the HER2 binding SPA domain derivative of the invention, is the albumin binding domain of streptococcal protein G (SPG) (Nygren P-Å *et al* (1988) Mol Recogn 1:69-74). A fusion polypeptide between the HER2 binding SPA domain-related polypeptide and the albumin binding domain of SPG thus falls within the scope of the present invention. When the polypeptide according to the invention is administered to a human subject as a therapeutic agent or as a targeting agent, the fusion thereof to a moiety which binds serum albumin may prove beneficial, in that the half-life *in vivo* of such a fusion protein may likely prove to be extended as compared to the half-

life of the SPA domain related HER2 binding moiety in isolation (this principle has been described ~~e g~~ e.g., in WO91/01743).

Please replace the paragraph bridging page 11, line 18, to page 12, line 20, with the following amended paragraph:

Other options for the second and further moiety or moieties of a fusion polypeptide according to the invention include a moiety or moieties for therapeutic applications. In therapeutic applications, other molecules may also be coupled, covalently or non-covalently, to the inventive polypeptide by other means. Non-limiting examples include enzymes for "ADEPT" (antibody-directed enzyme prodrug therapy) applications using the polypeptide according to the invention for direction of the effector enzyme (~~e g~~ e.g., carboxypeptidase); proteins for recruitment of effector cells and other components of the immune system; cytokines, such as IL-2, IL-12, TNFα, IP-10; procoagulant factors, such as tissue factor, von Willebrand factor; toxins, such as ricin A, Pseudomonas exotoxin, calcheamicin, maytansinoid; toxic small molecules, such as auristatin analogs, doxorubicin. Also, the above named additional amino acids (notably hexahistidine tag, cysteine),provided with the aim of coupling chelators for radiosotopes to the polypeptide sequence,

are contemplated, in order to easily incorporate radiating nuclides for diagnosis (~~e g~~ e.g., ^{68}Ga, ^{76}Br, ^{111}In, ^{99}Tc, ^{124}I, ^{125}I) or therapy (~~e g~~ e.g., ^{90}Y, ^{131}I, ^{211}At).

Please replace the paragraph brdiging page 17, line 17, to page 18, line 10, with the following amended paragraph:

However, the polypeptide according to the invention may also be produced by other known means, including chemical synthesis or expression in different prokaryotic or eukaryotic hosts, including plants and transgenic animals. When using chemical polypeptide synthesis, any of the naturally occurring amino acid resides in the polypeptide as described above may be replaced with any corresponding, non-naturally occurring amino acid residue or derivative thereof, as long as the HER2 binding capacity of the polypeptide is not substantially compromised. The binding capability should at least be retained, but replacement with a corresponding, non-naturally occurring amino acid residue or derivative thereof may actually also serve to improve the HER2 binding capacity of the polypeptide. Also, the incorporation of a non-naturally occurring amino acid may be performed in order to provide a site for alternative coupling of molecules (~~e g~~ e.g., labels, effectors, chelators etc) to the

HER2 binding polypeptide. Non-classical amino acids, or synthetic amino acid analogs, include, but are not limited to, the D-isomers of the common amino acids, α-amino isobutyric acid, 4-amino butyric acid, 2-amino butyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoroamino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid residues can be present in D or L form.

Please replace the paragraph at page 18, lines 11-26, with the following amended paragraph:

The present invention also concerns different aspects of using the above-described HER2 binding polypeptide, as well as various methods for treatment, diagnosis and detection in which the polypeptide is useful due to its binding characteristics. When referring to the "HER2 binding polypeptide" in the following description of these uses and methods, this term is intended to encompass the HER2 binding polypeptide alone, but

also all those molecules based on this polypeptide described above that ~~e g~~ e.g., constitute fragments thereof and/or incorporate the HER2 binding polypeptide as a moiety in a fusion protein and/or are conjugated to a label or therapeutic agent and/or are provided with additional amino acid residues as a tag for other purposes. As explained above, such fusion protein, derivatives, fragments etc form a part of the present invention.

Please replace the paragraph bridging page 20, line 35, to page 21, line 20, with the following amended paragraph:

Another aspect of the present invention is the use of the HER2 binding polypeptide as described herein for the detection of HER2 in a sample. For example, such detection may be performed with the aim of diagnosing disease states characterized by overexpression of HER2. The detection of HER2 presence in a sample may be performed *in vitro or in vivo*. A preferred option for *in vivo* diagnosis is the use of positron emission tomography, PET. The sample in question may ~~e g~~ e.g., be a biological fluid sample or a tissue sample. A common method, in use today with antibodies directed against HER2, which method may be adapted for use with the HER2, which method may be adapted for use with the HER2 binding polypeptides of the

present invention, is histochemical detection of HER2 presence used for identification of HER2 protein overexpression in fresh, frozen, or formalin-fixed, paraffinembedded tissue samples. For the purposes of HER2 detection, the polypeptide according to the invention may again be used as part of a fusion protein, in which the other domain is a reporter enzyme or fluorescent enzyme. Alternatively, it may be labeled with one or more fluorescent agent(s) and/or radioactive isotope(s), optionally via a chelator. Suitable radioactive isotopes include ^{68}Ga, ^{76}Br, ^{111}In, ^{99}Tc, ^{124}I and ^{125}I.

Please replace the paragraph bridging page 29, line 31 to page 30, line 9, with the following amended paragraph:

Sequencing of the DNA from the clones isolated ac- cording to the procedure above was performed with the ABI PRISM, BigDye Terminator v2.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations. Plasmids were prepared and DNA encoding the Affibody molecules was sequenced using the oligonucleotides RIT-27 (5'-GCTTCCGGCTCGTATGTTGTGTG- 3') (SEQ ID NO:80) and the biotinylated NOKA-2 (5'-biotin-CGGAACCAGAGCCACCACCGG-3') (SEQ ID NO:81). The sequences were analyzed on an ABI PRISM 3700 Genetic Analyser

(Applied Biosystems). From the 53 clones previously selected, several clones were found to encode the same amino acid sequence. Taking these degeneracies into account, four sequences of Affibody molecules expressed by clones selected in the ELISA binding assay are given in Figure 1 ($Z_{HER2\ A-D}$). and identified in the sequence listing as SEQ ID NO: 2-5.

Please replace the paragraph bridging page 35, line 26, to page 36, line 15, with the following amended paragraph:

A Biacore® 2000 instrument (Biacore AB) was used for real-time biospecific interaction analysis (BIA). A recombinant extracellular domain of HER2 (HER2-ECD), diluted in 10 mM NaAc, pH 4.5, was immobilized (about 2200 RU) on the carboxylated dextran layer of one flow-cell surface of a CM5 sensor chip (research grade) (BR-1000-14, Biacore AB) by amine coupling according to the manufacturer's instructions. Another flow-cell surface was activated and deactivated, to serve as a reference surface. For the Z_{HER2} sample, the buffer was changed to HBS (5 mM HEPES, 150 mM NaCl, 3.4mM EDTA, 0.005% surfactant P20,pH 7.4) be gel filtration using a NAP™-10 column, ~~according to the manufacturer's protocol~~ according to the manufacturer's protocol (Amersham Biosciences), and the sample was thereafter filtrated

(0.45 μm, Millipore, Billerica, MA). Binding analyses were performed at 25 °C, and HBS was used as running buffer. For all Biacore analyses, the samples were run in duplicates in random order, and after each injection the flow cells were regenerated by the injection of 10 mM HCl.

Please replace the paragraph bridging page 57, line 30, to to page 58, line 9, with the following amended paragraph:

Two days in advance, ~~100 000~~ 100,000 SKBR-3 cells, characterized by HER2 overexpression, were seeded in 3 cm dishes. 60 μg His_6-$(Z_{HER2\ A})_2$ polypeptide was labeled with ^{211}At produced in the Scanditronix MC32 cyclotron of the Copenhagen University Hospital and purified in the laboratory of Jörgen Carlsson, Uppsala University, Uppsala, Sweden. An approximate 1:1 and 5:1 molar ratio of ^{211}At-$(Z_{HER2\ A})_2$ molecules per cell receptor were added to three dishes each. Three additional dishes were supplied with the 5:1 concentration of ^{211}At-$(Z_{HER2\ A})_2$ but also a 500 times access of unlabeled His_6-$(Z_{HER2\ A})_2$ to block the binding sites and thereby estimate effects of unspecific irradiation. Cells were incubated with ^{211}At-$(Z_{HER2\ A})_2$ for 24 hours in 37 °C. After 24 hours of incubation, all cells were

washed and supplied with new, fresh medium. They were then monitored for growth once a week for about two months.

Please replace the paragraph bridging page 61, line 27, to page 62, line 13, with the following amended paragraph:

Sequencing of DNA encoding the Z_{HER2} variants analyzed in the ABAS-ELISA was performed with ABI PRISM; dGTP, BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations, using the biotinylated oligonucleotide AFFI-72 (5'-biotin-CGGAACCAGAGCCACCACCGG) (SEQ ID NO:82). The sequences were analyzed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems). The sequence analysis resulted in 130 unique sequences. Sequences originating from Z_{HER2} variants that exhibited binding in the ABAS ELISA experiment, as evidenced by an absorbance value that is at least two times higher than the value of the negative control, are presented in Figure 1 and identified in the sequence listing as SEQ ID NO: 6-76. The nomenclature of these HER2 binding Z variants is as follows. The variants isolated from the first plate of the ELISA experiment (Figure 29A) are denoted $Z_{HER2\ 1NN}$, where NN corresponds to the number of the well in the first plate in which that

particular polypeptide variant was analyzed. The variants isolated from the second plate of the ELISA experiment (Figure 29B) are denoted $Z_{HER2:2NN}$, where NN corresponds to the number of the well in the second plate in which that particular polypeptide variant was analyzed.

Please replace the paragraph at page 63, lines 26-37, with the following amended paragraph:

A comprehensive sequence analysis was performed of clones obtained after the third and fourth round from the selection described in Example 1. DNA was sequenced using the ABI PRISM dGTP, BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations and an ABI PRISME 3100 Genetic Analyser (Applied Biosystems). The biotinylated oligonucleotide AFFI-72 (5'-biotin- CGGAACCAGAGCCACCACCGG) (SEQ ID NO:82) was used as a primer. Sequence analysis revealed 11 new polypeptide sequences, i e clones that had not been found in the study described in Example 1.